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The Mode of Action of Isoxaflutole II. Characterization of the Inhibition of Carrot 4-Hydroxyphenylpyruvate Dioxygenase by the Diketonitrile Derivative of Isoxaflutole[*1, *2]

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Abstract

Isoxaflutole (*5-cyclopropylisoxazol-4-yl 2-mesyl-4-trifluromethylphenyl ketone*) is a novel herbicide for broadleaf and grass weed control in corn and sugarcane which acts by inhibiting the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD). In plants and soil, isoxaflutole is rapidly converted to a diketonitrile derivative (DKN) which is the active herbicide principle. The kinetics of inhibition of carrot HPPD*in vitro* by the DKN showed that it is a potent tight-binding inhibitor (IC_{50} value 4.9 ± 0.2 nM) exhibiting a time-dependent interaction with the enzyme in its ferrous state. Additional investigations provided evidence that the DKN is a competitive inhibitor that rapidly inactivates the enzyme (with a constant rate of association of 0.2 ± 0.004 $\mu M^{-1}s^{-1}$) by forming a reversible complex that releases slowly the inhibitor in an unmodified form. The decarboxylation coupled with reduction of molecular oxygen is generally accepted as the first enzymatic event of the HPPD-catalyzed reaction which occurs

as 4-hyroxyphenylpyruvate binds to the internal iron of protein*via* its ketoacid function. The DKN of isoxaflutole presents a β-(1,3)-diketone moiety, a delocalized π system which can mimic the ketoacid functionality of the substrate and which is also well known for its iron-chelating properties. Since this inhibitor competes with the substrate for binding, it is highly probable that it chelates the ferrous iron in the active site strongly by forming a stable ion-dipole charge transfer complex that resembles the initial substrate-iron complex or an early reaction intermediate. The slow release of the inhibitor in an unmodified form also suggests that the molecular oxygen activation due to ferrous iron generating a powerful oxidant as the inhibitor-enzyme complex forms is probably not occurring.

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*2 HPPD, 4-hydroxyphenylpyruvate dioxygenase; HPPA, 4-hydroxyphenylpyruvate; HGA, homogentisate; DKN, diketonitrile of isoxaflutole; NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl)-cyclohexane-1, 3-dione.

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